

a<sup>1</sup> It is an object of the present invention to provide purified stable UVDE (Uve1p), polypeptide fragments which retain high levels of activity, particularly those from the *Schizosaccharomyces pombe* enzyme. In a specific embodiment, the polypeptide fragment is Δ228-UVDE, which contains a 228 amino-acid deletion of the N-terminal region of the *S. pombe* *uve1* + gene product; a second specific embodiment is the fusion protein GST-Δ228-UVDE. The DNA sequence encoding GST-full-length UVDE from *S. pombe* is given in SEQ ID NO:1. The deduced amino acid sequence of full-length UVDE is given in SEQ ID NO:2. The DNA sequence encoding Δ228-UVDE is given in SEQ ID NO:3. The deduced amino acid sequence of Δ228-UVDE is given in SEQ ID NO:4. The DNA coding sequence and deduced amino acid sequence for GST-Δ228-UVDE are given in SEQ ID NO:5 and SEQ ID NO: 6, respectively. Also encompassed within the present invention are truncated UVDE proteins wherein the truncation is from about position 100 to about position 250 with reference to SEQ ID NO:2, and wherein the truncated proteins are stable in substantially pure form.

**Please amend the second full paragraph on page 6 to read as follows:**

a<sup>2</sup> Another embodiment of the invention features a hybridoma which produces an antibody having specific binding affinity to a UVDE polypeptide fragment. By "hybridoma" is meant an immortalized cell line which is capable of secreting an antibody, for example a Δ228-UVDE specific antibody. In preferred embodiments, the UVDE specific antibody comprises a sequence of amino acids that is able to specifically bind Δ228-UVDE. Alternatively, a GST-tag specific antibody or labeled ligand could be used to determine the presence of or quantitate a GST-Δ228-UVDE polypeptide, especially in formulations *ex vivo*.

**Please amend the paragraph bridging pages 11-12 to read as follows:**

a<sup>3</sup> Fig. 10A shows cleavage of an oligonucleotide substrate containing an AP site by Uve1p. To investigate if Uve1p was capable of cleaving an abasic site in a hydrolytic manner, we prepared a 5' end-labeled (\*) abasic substrate, AP-37mer, and incubated this substrate with buffer alone (lane 1), *E. coli* endonuclease III (AP lyase, lane 2), affinity-purified GΔ228-Uve1p and Δ228-Uve1p (2 μg of each) (lanes 3 and 4), extracts of cells over-expressing GΔ228-Uve1p (5 μg) (lane

5), *E. coli* endonuclease IV (hydrolytic AP endonuclease, lane 6) and purified recombinant GST (2  $\mu$ g) (lane 7). Fig. 10B demonstrates competitive inhibition of AP site recognition and cleavage. To demonstrate that the products generated are as a result of Uve1p-mediated cleavage at the AP site, AP-37mer was incubated with buffer alone (lane 1), *E. coli* endonuclease IV (lane 2), and affinity-purified G $\Delta$ 228-Uve1p (2  $\mu$ g) (lane 3) with 10X and 40X unlabeled cs-CPD-30mer (lanes 4 and 5, respectively) and 10X and 40X unlabeled UD-37mer (lanes 6 and 7, respectively). Arrows **a** and **b** indicate the primary and secondary Uve1p-mediated cleavage products, respectively. Arrow **uc** indicates the uncleaved substrate. A portion of the sequence of the AP substrate is shown at the bottom of the figure. S corresponds to deoxyribose and p corresponds to phosphate. The location of the cleavage sites of endonuclease III (E<sub>III</sub>) and endonuclease IV (E<sub>IV</sub>) are also indicated. For simplicity the complementary strand is omitted from the figure.

**Please amend the paragraph bridging pages 22-23 to read as follows:**

Uve1p isolated from *S. pombe* was first described as catalyzing a single ATP-independent incision event immediately 5' to the UV photoproduct, and generating termini containing 3' hydroxyl and 5' phosphoryl groups (Bowman et al. [1994] *Nucl. Acids Res.* **22**:3026-3032). The purified G $\Delta$ 228-Uve1p,  $\Delta$ 228-Uve1p and crude cell lysates of recombinant G-Uve1p and G $\Delta$ 228-Uve1p make an incision directly 5' to CPDs similar to that observed with the native protein. In this study, we have used both 5' and 3' end-labeled duplex CPD-30mer (cs-CPD-30mer) to demonstrate the ability of Uve1p to cleave a CPD-containing substrate at two sites (Fig. 6A-6B). The primary product (arrow **a**) accounted for approximately 90% of the total product formed and resulted from cleavage immediately 5' to the damage. The second incision site was located one nucleotide upstream and yielded a cleavage product (arrow **b**), which represented the remaining 10% of the product formed. This minor product is one nucleotide shorter or longer than the primary product depending on whether 5' or 3' end-labeled substrate is being examined. The same cleavage pattern was observed for each different Uve1p preparation used: i.e., crude extracts of cells expressing G $\Delta$ 228-Uve1p, affinity-purified G $\Delta$ 228-Uve1p and  $\Delta$ 228-Uve1p (Fig. 2A and 2B, lanes 2, 3 and 4 respectively), as well as extracts of cells expressing GST-Uve1p. No cleavage products were observed when the cs-CPD-30mer substrates were incubated with buffer

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only, or purified recombinant GST prepared and affinity-purified in an identical manner to the purified Uve1p proteins ( Fig. 6A, 6B, lanes 1 and 5 respectively). This control eliminates the possibility that these DNA strand scission products are formed as a result of the presence of trace amounts of non-specific endonuclease contamination. Uve1p recognizes a duplex cs-CPD-containing oligonucleotide substrate and cleaves this substrate at two sites. The primary site, responsible for 90% of the product, is immediately 5' to the damage and the secondary site (accounting for the remaining 10% of product), is one nucleotide 5' to the site of damage.

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**Please amend the full paragraph at page 29, lines 6-25, as follows:**

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Uve1p has been shown to incise DNA containing CPDs and 6-4PPs directly 5' to the photoproduct site generating products containing 3'-hydroxyl and 5'-phosphoryl groups [Bowman et al. (1994) *supra*]. We examined whether similar 3' and 5' termini were produced following Uve1p-mediated cleavage of base mismatch-containing substrates. DNA strand scission products generated by GΔ228-Uve1p cleavage of 3' end-labeled oligo \*CX/AY-31mer (CX strand labeled, Table 1B) were further treated with calf intestinal phosphatase (CIP) which removes 5' terminal phosphoryl groups from substrate DNA. The major sites of Uve1p-mediated DNA cleavage relative to the base mispair site were found to be at positions 0 and -1 (Fig. 11A, lane 2). CIP treatment of these DNA cleavage products resulted in species that had retarded electrophoretic mobilities compared to non-CIP-treated DNA cleavage products, indicating a decrease in charge corresponding to removal of 5' terminal phosphoryl groups (Fig. 11A, lanes 2 and 3). In addition, GΔ228-Uve1p mismatch endonuclease-generated DNA cleavage products were resistant to phosphorylation by polynucleotide kinase, an expected result if the 5' termini already contain phosphoryl groups (Fig. 11A, lane 4). Electrophoretic mobility shift analysis utilizing 5' end-labeled \*CX/AY-31mer, terminal deoxyribonucleotidyl transferase (TdT), and α<sup>32</sup>P-dideoxyATP (ddATP) resulted in addition of a single ddAMP to the 3' end of GΔ228-Uve1p-generated DNA cleavage products and indicates the presence of a 3'-hydroxyl terminus. These results show that the 3' and 5' termini of the products of GΔ228-Uve1p-mediated cleavage of substrates containing single base mismatches are identical to those generated following cleavage of substrates containing

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